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# Host phenotype characteristics and *MC1R* in relation to early-onset basal cell carcinoma

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## Abstract

Basal cell carcinoma (BCC) incidence is increasing, particularly among adults under age 40. Pigment-related characteristics are associated with BCC in older populations, but epidemiologic studies among younger individuals and analyses of phenotype-genotype interactions are limited. We examined self-reported phenotypes and melanocortin 1 receptor gene (*MC1R)* variants in relation to early-onset BCC. BCC cases (n=377) and controls with benign skin conditions (n=390) under age 40 were identified through Yale's Dermatopathology database. Factors most strongly associated with early-onset BCC were skin reaction to first summer sun for one hour [severe sunburn vs. tan odds ratio (OR)=12.27, 95% confidence interval (CI)=4.08–36.94] and skin color (very fair vs. olive OR=11.06, 95% CI=5.90–20.74). Individuals with two or more *MC1R* non-synonymous variants were 3.59 times (95% CI=2.37–5.43) more likely to have BCC than those without non-synonymous variants. All host characteristics and *MC1R* were more strongly associated with multiple BCC cases status (37% of cases) than single BCC case status. *MC1R*, number of moles, skin reaction to first summer sun for one hour, and hair and skin color were independently associated with BCC. BCC risk conferred by *MC1R* tended to be stronger among those with darker pigment phenotypes, traditionally considered to be at low-risk of skin cancer.

## Introduction

Basal cell carcinoma (BCC), which accounts for 80% of non-melanoma skin cancers (NMSCs), is the most common cancer in the US, with more than two million BCCs diagnosed annually (Rogers *et al.*, 2010; ACS, 2011). While BCC is unlikely to metastasize and is associated with low mortality, morbidity associated with this disease is quite high. In



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**Conflict of Interest**

The authors state no conflict of interest.

1992 among US Medicare beneficiaries, NMSC ranked among the top five most costly cancers to treat (Housman *et al.*, 2003). Newer data indicate from 1992 to 2006 in the Medicare population, there was a 77% increase in the total number of skin cancer-related procedures (93.7% NMSC), due to an increase in the number of individuals with these malignancies (Rogers *et al.*, 2010). In recent decades, BCC incidence has increased (Arits *et al.*, 2011; Bath-Hextall *et al.*, 2007; Birch-Johansen *et al.*, 2010; Doherty *et al.*, 2010; Flohil *et al.*, 2011; Karagas *et al.*, 1999; Levi *et al.*, 2001), with notable increases among adults under the age of 40, particularly women (Bath-Hextall *et al.*, 2007; Birch-Johansen *et al.*, 2010; Christenson *et al.*, 2005).

Ultraviolet (UV) radiation is the primary environmental etiologic factor for BCC, yet intrinsic or host factors, including pigment-related characteristics, are also likely to play a role in carcinogenesis in conjunction with UV (reviewed in (Dessinioti *et al.*, 2010; Madan *et al.*, 2010)). Among pigment-related factors, the melanocortin 1 receptor gene (*MC1R*), which encodes a protein that binds melanocyte-stimulating hormone and regulates skin and hair pigmentation (Valverde *et al.*, 1995), has received considerable attention and has been associated with an increased risk of melanoma and BCC (reviewed in (Scherer and Kumar, 2010)). Even though *MC1R* variants are related to light pigmentation phenotypes (Bastiaens *et al.*, 2001; Box *et al.*, 2001; Dwyer *et al.*, 2004; Han *et al.*, 2006; Kanetsky *et al.*, 2004; Kennedy *et al.*, 2001; Koppula *et al.*, 1997; Naysmith *et al.*, 2004; Palmer *et al.*, 2000; Smith *et al.*, 1998; Valverde *et al.*, 1995), there seems to be an effect of genotype independent of phenotype on both BCC (Bastiaens *et al.*, 2001; Box *et al.*, 2001; Dwyer *et al.*, 2004; Han *et al.*, 2006; Liboutet *et al.*, 2006; Scherer *et al.*, 2008) and melanoma (Dwyer *et al.*, 2004; Kanetsky *et al.*, 2010; Kennedy *et al.*, 2001; Landi *et al.*, 2005; Palmer *et al.*, 2000). These findings, in combination with other emerging evidence from epidemiologic, clinical, and basic science research, indicate BCC may be more similar to melanoma than squamous cell carcinoma (SCC) in etiology (Dessinioti *et al.*, 2010; Madan *et al.*, 2010).

BCC has been relatively understudied in epidemiologic research because it is not reported to most cancer registries. Thus far, two studies provided an intriguing glimpse at risk factors for early-onset BCC, but these had small sample sizes; 30 cases (Boyd *et al.*, 2002) and 25 cases (Bakos *et al.*, 2011). Due to the limited understanding of early-onset BCC etiology, we conducted a case-control study in Connecticut among individuals under age 40 investigating lifestyle, environmental, and genetic factors. The rationale for this study was multifold and included increasing incidence among young people, the opportunity to evaluate genetic factors in a genetically enriched population, the potential for younger individuals to better recall early-life exposures, and a growing prevalence of indoor (IARC, 2007) and outdoor tanning.

Here, we describe the design of the Yale Study of Skin Health in Young People and the associations between host phenotype characteristics and *MC1R* in relation to early-onset BCC. We also evaluated potential variation in the association between *MC1R* and BCC by phenotype.

## Results

Sixty-nine percent of the 767 participants were female (257 cases, 274 controls). The mean age at skin biopsy in cases was 35.1 years (SD=4.6) and 34.7 years (SD=5.5) in controls. Among cases, 54.1% (n=204) had the referent BCC on the head or neck, followed by 101 (26.8%) with a trunk BCC, and 72 (19.1%) with a BCC on an extremity. Approximately 37% (n=140) of cases had two or more BCCs under age 40.

All phenotype characteristics and *MC1R* were significantly associated with early-onset BCC, with lighter pigment phenotypes at greater risk (Table 1). The most pronounced risk factor was skin reaction to first summer sun of the season; those who experienced severe sunburn and blistering were 12.27 (95% CI=4.08–36.94) times more likely to have BCC than those who turned brown/tanned with no burning. Skin color was another strong risk factor; individuals with very fair skin were 11.06 (95% CI=5.90–20.74) times more likely to have BCC than those with olive skin. In a sensitivity analysis, excluding the top three control conditions one at a time from the control group did not impact risk estimates for all exposures (data not shown). Controlling for indoor and outdoor UV exposures did not alter associations for the characteristics of interest (data not shown).

We detected 35 *MC1R* variants (Supplemental Table 1 Online). Individuals with one *MC1R* non-synonymous variant were 93% more likely than those without non-synonymous variants to have BCC, with a stronger association for individuals with two or more non-synonymous *MC1R* variants (OR=3.59, 95% CI=2.37–5.43) (Table 1). Risk was elevated for both “major” and “minor” red hair variants (Table 2).

All host characteristics were associated with both single and multiple BCC case status, but the magnitude of the risk estimates for multiple BCC was much greater (Table 1). One of the most pronounced differences was for skin color. While very fair skin as compared to olive skin was associated with a 6.62 increased risk of single BCC, the OR for multiple BCC was almost 5.5 times greater (OR=36.07, 95% CI=8.95–161.94).

Participants with lighter pigment characteristics, less ability to tan, and more freckles were more likely to have at least one non-synonymous *MC1R* variant as compared to those with darker phenotypes (Supplemental Table 2 Online).

In the mutually adjusted model, hair and skin color, *MC1R*, moles, and skin reaction to first summer sun were independently associated with BCC (Table 3). Very fair skin was associated with a 4.48 fold independent increased risk of BCC compared to olive skin (OR=4.48, 95% CI=2.21–9.09) and individuals with two or more non-synonymous variants had a 91% independent increased risk compared to those with no variants (OR=1.91, 95% CI=1.20–3.03).

While there was no evidence of significant interactions between phenotypes and *MC1R* in relation to BCC risk, we observed some general patterns in risk across strata (Table 4). The association between *MC1R* and BCC was stronger among individuals with darker phenotypes including, darker eye and skin color, fewer moles and freckles, and tanning rather than burning with sun exposure.

## Discussion

In this case-control study of early-onset BCC, host phenotype characteristics of lighter pigmentation and inability to tan, as well as *MC1R* were independently associated with increased disease risk. To our knowledge, a large-scale epidemiologic study focused exclusively on BCC among young adults has not been previously reported. In our unique population, the magnitudes of risk associated with phenotype characteristics often associated with BCC were generally magnified as compared to studies in older individuals (Dessinioti *et al.*, 2010; Hogan *et al.*, 1989; Kiiski *et al.*, 2010; Maia *et al.*, 1995; Naldi *et al.*, 2000; Vitasa *et al.*, 1990; Zanetti *et al.*, 1996). Although BCC is relatively rare in young people, 37% of our cases had two or more BCCs under the age of 40, and the association with each of our exposures was much stronger for these cases.

Our finding of a nearly two-fold increase in BCC risk for one non-synonymous *MC1R* variant and a 3.6 fold increase for two non-synonymous variants is in agreement with other BCC studies (Bastiaens *et al.*, 2001; Box *et al.*, 2001; Dwyer *et al.*, 2004; Han *et al.*, 2006; Scherer *et al.*, 2008). Yet, as we hypothesized, the magnitude of risk we observed was greater than in studies of older adults, where risk estimates have been less than or equal to 2.6 (Bastiaens *et al.*, 2001; Box *et al.*, 2001; Dwyer *et al.*, 2004; Han *et al.*, 2006; Scherer *et al.*, 2008). Of note, one small case-control study with a heterogeneous case group enrolled on the basis of having either familial BCC, multiple BCC, BCC with another cancer, or BCC before age 40, observed a seven-fold increased risk of BCC with two *MC1R* variants (Liboutet *et al.*, 2006). Similar to our findings, *MC1R* variants have been associated with lighter pigment phenotypes in numerous studies (Bastiaens *et al.*, 2001; Box *et al.*, 2001; Dwyer *et al.*, 2004; Han *et al.*, 2006; Kanetsky *et al.*, 2004; Kennedy *et al.*, 2001; Koppula *et al.*, 1997; Naysmith *et al.*, 2004; Palmer *et al.*, 2000; Smith *et al.*, 1998; Valverde *et al.*, 1995).

The independent associations with early-onset BCC for *MC1R*, hair and skin color, moles, and skin reaction are in line with several studies of BCC (Bastiaens *et al.*, 2001; Box *et al.*, 2001; Dwyer *et al.*, 2004; Han *et al.*, 2006; Liboutet *et al.*, 2006; Scherer *et al.*, 2008) and melanoma (Dwyer *et al.*, 2004; Kanetsky *et al.*, 2010; Kennedy *et al.*, 2001; Landi *et al.*, 2005; Palmer *et al.*, 2000). That *MC1R* remained an independent risk factor suggests variants in this gene contribute to BCC pathogenesis through mechanisms besides pigmentation. As a potential tumor initiator, impairment of *MC1R* function leads to synthesis of pheomelanin, which acts as a free radical generator and may cause oxidative DNA damage on top of the UVB-induced damage typically associated with sunlight exposure to fair skin (Scherer and Kumar, 2010). Furthermore, mouse studies show an effect of *MC1R* genotype on production of premalignant clones in the absence of any melanin pigment, suggesting a mechanism separate from pigment modulation (Robinson *et al.*, 2010). A role in tumor progression through regulation of cytokines and their associated receptors, such as NF-kB, has been suggested (Eves *et al.*, 2003; Getting, 2006). There is a complex interplay between NF-kB regulation and the ability of tumor cells to escape immune surveillance and invade surrounding tissues.

The association of pigment phenotypes independent of *MC1R* genotype points toward the involvement of other pigment-related genes in BCC risk. *TYR*, *ASIP*, and *SLC45A2* have been identified in other BCC studies (Gudbjartsson *et al.*, 2008; Nan *et al.*, 2009; Scherer and Kumar, 2010; Stacey *et al.*, 2009).

We found that *MC1R* was more strongly associated with early-onset BCC among those with darker phenotypes. Several other BCC studies have evaluated this genotype-phenotype interaction, but results have been inconsistent. One study observed no clear variation in the association of *MC1R* and BCC risk by hair or skin color (Han *et al.*, 2006), while others found an increased risk in those with darker hair and skin, but opposite patterns for eye color (Liboutet *et al.*, 2006), or suggestive increased risk among individuals with the lightest skin (Bastiaens *et al.*, 2001; Scherer *et al.*, 2008). One study of BCC (Dwyer *et al.*, 2004) and several of melanoma (Dwyer *et al.*, 2004; Ichii-Jones *et al.*, 1998; Kanetsky *et al.*, 2010; Landi *et al.*, 2005; Palmer *et al.*, 2000) had findings similar to ours.

Our findings, in conjunction with research in melanoma, may have applicability in primary prevention. Among participants with the darkest pigment phenotypes, the estimated etiologic fractions for carrying two or more non-synonymous *MC1R* variants ranged from a low of 12% among individuals with no freckles on the face to a high of 28% among individuals with brown eyes. Because people with darker phenotypes are also at risk of skin cancer, sun protection interventions may need to be broadened to include these individuals who would otherwise consider themselves low-risk.

Our study had several strengths including extensive self-report phenotype data from a face-to-face interview and *MC1R* sequencing for nearly all participants. Importantly, the laboratory was blinded to case-control status and interviewers were blinded to case-control status until the end of the interview. Utilizing a centralized dermatopathology facility serving many dermatologists in Connecticut enabled us to identify controls most likely to constitute the source population of our cases; that is, young people who see a dermatologist for a skin lesion. Because our controls had undergone a skin biopsy, this may have reduced differential reporting by case status, as our controls may have been more sensitive to exposures concerning skin health than general population controls. Our results were robust in sensitivity analyses removing specific control conditions, indicating associations were not driven by inclusion of one benign condition.

As in any case-control study, selection bias is a potential concern. Another potential limitation is related to possible misclassification within participant self-reported measures of phenotype, as we did not have more objective measures of pigment characteristics, such as clinician assessment or spectroscopy, but this is most likely to be non-differential. Finally, although controls were seen by a dermatologist for a benign skin condition, we did not know if a complete skin examination was performed. Therefore, controls could have possibly had a BCC; however, the likelihood of this is low in our young sample.

In summary, several host phenotype characteristics and *MC1R* were strongly and independently related to early-onset BCC. In this young population, the associations between the exposures of interest and disease risk were more pronounced for multiple BCC,

and the relationship between *MC1R* and BCC was stronger among individuals with darker pigmentation phenotypes. Even persons with darker pigment phenotypes, traditionally considered to be low risk of skin cancer, were at substantial risk of early-onset BCC if they had *MC1R* variants. To our knowledge a large scale epidemiologic investigation of these characteristics in relation early-onset BCC is previously unreported, so our results need confirmation in other populations.

## Materials and Methods

### Study Design

The Yale Study of Skin Health in Young People was conducted in Connecticut between July 2007 and December 2010. BCC cases diagnosed between July 1, 2006 and September 30, 2010 were identified through Yale University's Dermatopathology database. Approximately two-thirds of dermatologists in Connecticut send their biopsied tissue to Yale for dermatopathologic evaluation. Potential controls were randomly sampled from individuals in the database with a variety of minor benign skin conditions. To be eligible for the study, participants had to: be less than 40 years of age at the time of skin biopsy, reside in Connecticut, speak English, and themselves (or appropriate guardian for decisionally impaired individuals and those under age 18) be mentally and physically capable of completing study components. Yale University's Institutional Review Board approved the study and participants (or guardians) provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki Principles.

Potential participants were mailed a letter and study brochure. One of two study interviewers then contacted individuals by telephone and invited them to participate in the study. If the telephone number was not working (disconnected, wrong number, no number listed), another letter was mailed asking for updated contact information via telephone or mail. If the telephone number and mailing address were incorrect, updated contact information was sought periodically.

Among the 665 potentially eligible BCC cases identified during the study period, 17 (2.6%) were determined ineligible upon initial contact: 14 moved out of the state and 3 could not complete all study components (two non-English speakers, one severe illness). Of the remaining 648 individuals, 114 (17.6%) could not be contacted directly (no telephone number, non-working telephone number, only spoke to other person in household, left message only). Among the 534 cases we were able to directly reach and determine full eligibility, 145 (27.2%) declined to participate, resulting in 389 enrolled cases (participation rate=72.8%).

Cases were classified into single (only one BCC) or multiple (two or more BCCs) BCC under the age of 40 based on participant self-report and searching the Yale Dermatopathology database (records from 1990 on). A total of 242 (62.2%) cases had one BCC in the database and did not self-report a prior BCC and 120 (30.9%) cases had two or more BCCs in the database. The remaining 27 (6.9%) cases had one BCC in the database, but self-reported a prior BCC; these individuals were categorized as multiple cases, as this did not significantly alter risk estimates.

To determine control eligibility, two dermatologists reviewed skin conditions diagnosed during a one-year period in persons under age 40 in the Yale Dermatopathology database. A variety of diagnoses were determined ineligible for sampling, including skin cancers/precancers (e.g., melanoma, squamous cell carcinoma, T-cell lymphomas, actinic keratoses), potentially UV-related benign conditions (e.g., solar lentigo, abnormal nevus, erythematous conditions), dermal conditions treated with UV therapy (e.g., psoriasis) and pigment disorders (e.g., vitiligo).

Randomly sampled controls were frequency matched to BCC cases on age at biopsy (5 year age groups), gender, and biopsy site (head/neck, trunk, extremity). Among the 1,102 potentially eligible controls, 60 (5.4%) were found ineligible upon initial contact (39 moved out of state, 10 non-English speakers, 2 did not recall having a skin biopsy, 1 hearing impaired, 1 hospitalized) or during the interview (7 self-reported a BCC). Of the remaining 1,042 individuals, 288 (27.6%) could not be contacted directly. Among the 754 potential controls we could directly reach and determine full eligibility, 296 (39.3%) declined to participate and 458 controls enrolled (participation rate=60.7%). Controls had a variety of benign skin conditions. The three most common were cyst (16.4%), seborrheic keratosis (16.2%), and wart (11.4%). All other conditions were present in less than 10% of controls.

Participants completed an in-person face-to-face interview during which interviewers obtained information on sociodemographics, UV exposure (solar and artificial), personal and family medical history, and host phenotype characteristics including, self-reported eye color, skin color (inner upper arm), hair color (natural color), skin reaction to strong sunlight for the first time in the summer for one hour without sunscreen, skin reaction after repeated and prolonged exposure to sunlight, amount of freckles on the face (selected from a range of images), and number of moles on the back = 5 mm (using clear acetate size template) using a structured questionnaire. Interviewers were blinded to case-control status until the end of the interview, when personal history of cancer, including BCC, was queried.

Participants also completed several mailed self-administered questionnaires (residence history, outdoor jobs, attitudes toward sunless, outdoor, and indoor tanning). Interviewers collected buccal cells from 98.9% of participants using Oragene®•DNA 2mL saliva collection kits (DNA Genotek Inc.; Ontario, Canada; http://www.dnagenotek.com/index.html) at the end of the interview following the manufacturer's protocol, including rinsing the mouth with drinking water and then waiting five minute before collection.

## MC1R Sequencing and Variant Classification

Oragene kits were stored at room temperature until processed. DNA was isolated based on the manufacturer's protocol. Laboratory personnel were blinded to case-control status.

The *MC1R* gene was PCR amplified as a single 1.3 kb fragment. Each 25 μl PCR reaction contained 25–50 ng of DNA; 200 μmol/L dNTPs; 5 μmol/L of each primer, 5′-ACTAAGCAGGACACCTGGAG-3′ and 5′-TCTTTAGGAGCCTGAGGTTG-3′; PC2 buffer (50 mM Tris-HCl pH 9.1, 16 mM ammonium sulfate, 3.5 mM MgCl2, and 150 mg/ml BSA; Ab Peptides, Inc.); 0.25 mmol/L spermidine; 0.125 units of Taq DNA polymerase (Amplitaq®, Roche); and 0.125 units of Taq Extender (Stratagene). PCR was

performed with an initial denaturation for two minutes at 97°C; followed by 35 cycles of denaturation at 96°C for 30 seconds, annealing at 66°C for 30 seconds, and extension for one minute at 72°C; and a final extension at 72°C for five minutes. PCR products were size fractionated on a 1.5% GPG/LETM (American Bioanalytical) agarose gel, stained with ethidium bromide, and photographed under ultraviolet light in order to confirm the presence of the correct PCR fragment.

PCR products were sequenced bidirectionally. 5 μl of the PCR products were treated with 20 units Exonuclease I (E.coli) (New England BioLabs) and two units Shrimp Alkaline Phosphatase (USB). Either 0.4 μmol/L of the forward primer, 5′-ACTAAGCAGGACACCTGGAG-3′, or the reverse primer 5′-GGTCACACAGGAACCAGACC-3′ were added. The sequencing was carried out at Yale University's W. M. Keck Facility using Applied Biosystems 3730 capillary instruments. The sequencing reactions utilized fluorescently-labeled dideoxynucleotides (Big Dye Terminators) and Taq FS DNA polymerase in a thermal cycling protocol. The sequence was analyzed using Sequencher 4.9 (Gene Codes Corporation) comparing the query sequence to the standard sequence with no variants in *MC1R* (NM_002386.3).

*MC1R* variants were classified into synonymous and non-synonymous variants. Non-synonymous variants were grouped into "major" and "minor" red hair variants (Box *et al.*, 1997; Kanetsky *et al.*, 2004; Valverde *et al.*, 1995). We then calculated the number of total non-synonymous variants within the *MC1R* coding region.

### Statistical Analysis

Analyses were limited to non-Hispanic Whites; 380 (97.7%) cases and 390 (85.2%) controls. Three BCC cases with Gorlin Syndrome, which predisposes individuals to multiple BCCs early in life (Gorlin and Goltz, 1960), were also excluded. Our analytic population consisted of 767 individuals (377 cases, 390 controls); three cases and three controls were under age 18 at enrollment.

Phenotype characteristics and *MC1R* (count of all non-synonymous variants within the gene, 0, 1, ≥ 2 variants) were treated as categorical variables. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) using multivariate logistic regression for all cases. Analyses were then restricted to cases with only one BCC (n=237) and then cases with two or more BCCs (n=140). We determined independent relationships using backward stepwise selection; retaining only exposures statistically significant at alpha=0.05, as well as gender, age, and body site. Phenotype-genotype interactions were tested with cross-product terms. Analyses were conducted using SAS Version 9.2 (Cary, NC).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **BCC** | basal cell carcinoma |
| **CI** | confidence interval |
| ***MC1R*** | melanocortin 1 receptor gene |
| **NMSCs** | non-melanoma skin cancers |
| **(OR)** | odds ratio |
| **SCC** | squamous cell carcinoma |
| **UV** | ultraviolet |

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**Table 1**

Odds ratios (OR) and 95% Confidence intervals (CIs) for association between host characteristics and *MC1R* and BCC in the Yale Study of Skin Health

| Characteristic | Cases, N=377 N[1] (%) | Control, N=390 N[1] (%) | All BCCs OR[2] (95% CI) | Single BCC, 237 cases OR[2] (95% CI) | Multiple BCC, 140 cases OR[2] (95% CI) |
|---|---|---|---|---|---|
| Eye color | | | | | |
| Brown | 86 (22.8) | 154 (39.5) | 1.00 | 1.00 | 1.00 |
| Hazel | 65 (17.2) | 72 (18.5) | 1.76 (1.14–2.72) | 1.66 (1.02–2.71) | 2.15 (1.08–4.27) |
| Green | 47 (12.5) | 38 (9.7) | 2.34 (1.40–3.91) | 1.94 (1.08–3.48) | 3.43 (1.64–7.17) |
| Blue/Grey | 179 (47.5) | 126 (32.3) | 2.60 (1.83–3.72) | 1.81 (1.21–2.71) | 5.20 (3.00–9.00) |
| Hair color | | | | | |
| Black/Dark brown | 101 (26.9) | 161 (41.3) | 1.00 | 1.00 | 1.00 |
| Light brown | 136 (36.2) | 155 (39.7) | 1.42 (1.01–2.01) | 1.28 (0.86–1.89) | 1.84 (1.09–3.10) |
| Blonde/Fair | 100 (26.6) | 63 (16.2) | 2.66 (1.76–4.01) | 2.15 (1.35–3.44) | 4.07 (2.29–7.22) |
| Red | 39 (10.4) | 11 (2.8) | 6.38 (3.08–13.22) | 3.74 (1.59–8.80) | 14.27 (6.11–33.32) |
| Skin color | | | | | |
| Olive | 15 (4.0) | 77 (19.7) | 1.00 | 1.00 | 1.00 |
| Fair | 213 (56.5) | 236 (60.5) | 4.78 (2.65–8.62) | 3.74 (1.98–7.04) | 11.39 (2.71–47.78) |
| Very fair | 149 (39.5) | 77 (19.7) | 11.06 (5.90–20.74) | 6.62 (3.34–13.10) | 36.07 (8.95–161.94) |
| Skin reaction with first summer sun exposure | | | | | |
| Turn brown, no sunburn | 6 (1.6) | 31 (8.0) | 1.00 | 1.00 | 1.00 |
| Mild sunburn followed by tan | 142 (37.7) | 200 (51.4) | 3.65 (1.47–9.07) | 3.34 (1.24–9.02) | 5.32 (0.70–40.51) |
| Painful sunburn peeling | 199 (52.8) | 144 (37.0) | 7.50 (3.00–18.72) | 5.10 (1.87–13.89) | 18.35 (2.44–138.16) |
| Severe sunburn blistering | 30 (8.0) | 14 (3.6) | 12.27 (4.08–36.94) | 7.34 (2.11–25.50) | 36.72 (4.36–309.37) |
| Skin reaction with prolonged sun exposure | | | | | |
| Very brown, deeply tanned | 39 (10.3) | 71 (18.2) | 1.00 | 1.00 | 1.00 |
| Moderately tanned | 169 (44.8) | 223 (57.2) | 1.38 (0.89–2.16) | 1.10 (0.67–1.80) | 2.42 (1.10–5.32) |
| Mildly tanned peeling tendency | 123 (32.6) | 78 (20.0) | 2.96 (1.81–4.85) | 1.97 (1.14–3.42) | 6.24 (2.76–14.11) |
| Freckled, no suntan | 46 (12.2) | 18 (4.6) | 4.89 (2.47–9.69) | 3.59 (1.67–7.69) | 10.19 (3.80–27.36) |
| *MC1R* non-synonymous variants | | | | | |
| 0 variants | 65 (17.3) | 131 (34.2) | 1.00 | 1.00 | 1.00 |
| 1 variant | 173 (46.0) | 175 (45.7) | 1.93 (1.33–2.80) | 1.76 (1.15–2.69) | 2.35 (1.33–4.14) |

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| Characteristic | Cases, N=377 N[1] (%) | Control, N=390 N[1] (%) | All BCCs OR[2] (95% CI) | Single BCC, 237 cases OR[2] (95% CI) | Multiple BCC, 140 cases OR[2] (95% CI) |
|---|---|---|---|---|---|
| ≥ 2 variants | 138 (36.7) | 77 (20.1) | 3.59 (2.37–5.43) | 2.93 (1.82–4.71) | 5.15 (2.84–9.32) |
| Moles ≥ 5 mm on back | | | | | |
| None | 173 (46.1) | 209 (53.6) | 1.00 | 1.00 | 1.00 |
| 0–4 | 137 (36.5) | 146 (37.4) | 1.17 (0.85–1.60) | 1.13 (0.79–1.61) | 1.27 (0.81–1.98) |
| 5–9 | 42 (11.2) | 24 (6.2) | 2.36 (1.36–4.12) | 1.62 (0.83–3.17) | 3.69 (1.90–7.17) |
| 10 or more | 23 (6.1) | 11 (2.8) | 3.00 (1.39–6.48) | 2.21 (0.86–5.64) | 4.43 (1.84–10.67) |
| Freckles on face | | | | | |
| None | 78 (20.7) | 139 (35.6) | 1.00 | 1.00 | 1.00 |
| Very few | 82 (21.8) | 112 (28.7) | 1.38 (0.92–2.07) | 1.31 (0.83–2.06) | 1.74 (0.90–3.37) |
| Few | 123 (32.6) | 93 (23.9) | 2.39 (1.61–3.55) | 1.80 (1.15–2.81) | 4.39 (2.38–8.08) |
| Some | 74 (19.6) | 36 (9.2) | 3.99 (2.42–6.58) | 2.96 (1.68–5.20) | 7.64 (3.78–15.46) |
| Many | 20 (5.3) | 10 (2.6) | 3.84 (1.69–8.76) | 1.50 (0.50–4.49) | 11.63 (4.43–30.54) |

[1] May not sum to total due to missing data.

[2] Adjusted for frequency matching study variables: age at diagnosis (continuous), body site (head, extremity, trunk), and gender.

## Table 2

Alternate classifications of *MC1R* variants in relation to BCC in the Yale Study of Skin Health. Referent group for all variables is individuals with no non-synonymous variants.

| Characteristic | Cases/Controls | OR[1] (95% CI) |
|---|---|---|
| No non-synonymous variants | 65/131 | 1.00 |
| *MC1R* "major" red hair variants[2] | | |
| 1 variant | 158/108 | 2.88 (1.94–4.27) |
| ≥ 2 variants | 31/11 | 5.85 (2.73–12.54) |
| *MC1R* alternate "major" red hair variants[3] | | |
| 1 variant | 147/95 | 2.97 (1.98–4.44) |
| ≥ 2 variants | 20/8 | 4.95 (2.04–12.00) |
| *MC1R* "minor" red hair variants[4] | | |
| 1 variant | 147/129 | 2.20 (1.55–3.11) |
| ≥ 2 variants | 34/30 | 3.09 (1.88–5.07) |
| 1 "major"[2] and 1 "minor" red hair variant | 63/31 | 3.95 (2.31–6.76) |

[1] Adjusted for age at diagnosis (continuous), body site (head, extremity, trunk), and gender.

[2] Includes D84E, R142H, R151C, I155T, R160W, and D294H.

[3] Includes R151C, R160W, and D294H.

[4] Includes V60L, V92M, and R163Q.

## Table 3

Mutually adjusted odds ratios (OR) and 95% confidence intervals (CIs) for the most parsimonious model of the association between host characteristics, *MC1R*, and BCC in the Yale Study of Skin Health

| Characteristic | Cases/Controls | Mutually adjusted OR[1] (95% CI) |
|---|---|---|
| Hair color | | |
| Black/Dark brown | 99/157 | 1.00 |
| Light brown | 135/151 | 1.31 (0.78–1.64) |
| Blonde/Fair | 100/63 | 1.63 (1.04–2.53) |
| Red | 39/11 | 2.74 (1.23–6.09) |
| Skin color | | |
| Olive | 15/74 | 1.00 |
| Fair | 211/232 | 2.75 (1.46–5.18) |
| Very fair | 147/76 | 4.48 (2.21–9.09) |
| *MC1R* non-synonymous variants | | |
| 0 variants | 65/130 | 1.00 |
| 1 variant | 172/175 | 1.41 (0.95–2.11) |
| ≥ 2 variants | 136/77 | 1.91 (1.20–3.03) |
| Moles ≥ 5 mm on back | | |
| None | 172/204 | 1.00 |
| 0–4 | 136/145 | 1.03 (0.73–1.44) |
| 5–9 | 42/22 | 1.93 (1.05–3.52) |
| 10 or more | 23/11 | 2.19 (0.98–4.90) |
| Skin reaction with first summer sun exposure | | |
| Turn brown, no sunburn | 6/31 | 1.00 |
| Mild sunburn followed by tan | 141/196 | 1.91 (0.73–5.03) |
| Painful sunburn peeling | 196/142 | 2.43 (0.90–6.58) |
| Severe sunburn blistering | 30/13 | 3.68 (1.11–12.23) |

[1] Adjusted for age at diagnosis (continuous), body site (head, extremity, trunk), gender and all other characteristics in table.

## Table 4

*MC1R* non-synonymous variants and BCC risk stratified by host characteristics in the Yale Study of Skin Health

| Characteristic | *MC1R* Variants | Cases/Controls | OR[1] (95% CI) | p for interaction[2] |
|---|---|---|---|---|
| **Eye color** | | | | 0.557 |
| Brown | 0 | 17/56 | 1.00 | |
| | 1 | 38/71 | 1.64 (0.82–3.29) | |
| | ≥ 2 | 31/23 | 4.37 (1.99–9.56) | |
| Hazel/Green | 0 | 17/34 | 1.00 | |
| | 1 | 52/48 | 2.06 (1.01–4.20) | |
| | ≥ 2 | 42/26 | 2.99 (1.38–6.47) | |
| Blue/Grey | 0 | 31/41 | 1.00 | |
| | 1 | 83/56 | 1.95 (1.08–3.53) | |
| | ≥ 2 | 65/28 | 3.25 (1.67–6.35) | |
| **Hair color** | | | | 0.670 |
| Black/Dark brown | 0 | 23/62 | 1.00 | |
| | 1 | 55/72 | 1.94 (1.05–3.58) | |
| | ≥ 2 | 22/23 | 2.62 (1.20–5.73) | |
| Light brown | 0 | 24/53 | 1.00 | |
| | 1 | 66/69 | 2.00 (1.09–3.67) | |
| | ≥ 2 | 46/30 | 3.12 (1.57–6.19) | |
| Blonde/Fair/Red | 0 | 18/16 | 1.00 | |
| | 1 | 51/34 | 1.23 (0.54–2.80) | |
| | ≥ 2 | 70/24 | 2.44 (1.05–5.65) | |
| **Skin color** | | | | 0.364 |
| Olive/Fair | 0 | 47/120 | 1.00 | |
| | 1 | 111/132 | 2.04 (1.32–3.14) | |
| | ≥ 2 | 69/55 | 3.38 (2.04–5.59) | |
| Very fair | 0 | 18/11 | 1.00 | |
| | 1 | 62/43 | 0.94 (0.39–2.25) | |
| | ≥ 2 | 69/22 | 1.81 (0.72–4.52) | |
| **Skin reaction to first summer sun exposure** | | | | 0.851 |
| Turn brown, no burn/Mild burn then tan | 0 | 38/96 | 1.00 | |
| | 1 | 69/98 | 1.64 (1.00–2.71) | |
| | ≥ 2 | 41/33 | 3.04 (1.64–5.61) | |
| Painful burn peeling/Severe burn blistering | 0 | 27/34 | 1.00 | |
| | 1 | 104/77 | 1.63 (0.90–2.96) | |
| | ≥ 2 | 97/44 | 2.64 (1.41–4.96) | |
| **Skin reaction to prolonged sun exposure** | | | | 0.299 |
| Very brown/Moderately tanned | 0 | 46/115 | 1.00 | |
| | 1 | 102/129 | 1.92 (1.24–2.99) | |
| | ≥ 2 | 59/45 | 3.49 (2.04–5.95) | |

| Characteristic | *MC1R* Variants | Cases/Controls | OR[1] (95% CI) | p for interaction[2] |
|---|---|---|---|---|
| Mildly tanned peeling/Freckled, no tan | 0 | 19/16 | 1.00 | |
| | 1 | 71/46 | 1.12 (0.51–2.45) | |
| | ≥ 2 | 79/32 | 1.81 (0.81–4.03) | |
| **Moles ≥ 5 mm on back** | | | | 0.496 |
| None | 0 | 28/74 | 1.00 | |
| | 1 | 85/91 | 2.54 (1.48–4.35) | |
| | ≥ 2 | 59/39 | 4.05 (2.20–7.47) | |
| 0–4 | 0 | 27/52 | 1.00 | |
| | 1 | 55/61 | 1.68 (0.92–3.07) | |
| | ≥ 2 | 55/33 | 3.31 (1.72–6.36) | |
| ≥ 5 | 0 | 10/5 | 1.00 | |
| | 1 | 33/23 | 0.51 (0.14–1.89) | |
| | ≥ 2 | 22/5 | 2.26 (0.49–10.46) | |
| **Freckles on face** | | | | 0.723 |
| None | 0 | 28/68 | 1.00 | |
| | 1 | 38/60 | 1.55 (0.83–2.92) | |
| | ≥ 2 | 12/8 | 4.33 (1.54–12.15) | |
| Very few | 0 | 18/38 | 1.00 | |
| | 1 | 38/50 | 1.73 (0.83–3.59) | |
| | ≥ 2 | 26/21 | 2.44 (1.06–5.61) | |
| Few/Some/Many | 0 | 19/25 | 1.00 | |
| | 1 | 97/65 | 1.88 (0.95–3.75) | |
| | ≥ 2 | 100/48 | 2.55 (1.26–5.15) | |

[1] Adjusted for age at diagnosis (continuous), body site (head, extremity, trunk), and gender.

[2] Based on inclusion of cross-product term in multivariate model.